Remarks / Arguments

Claims 22-24 are newly added. Claims 1-3, 17 and 22-24 are pending upon entry of this amendment.

Support for Amendments

The amendments provided herein are supported throughout the application as filed, including the specification. Exemplary support is provided for each amendment.

Claim 22 is newly added, depends from claim 1 and adds that the proteinaceous substances are antibodies capable of specific binding with antigen. Claim 23 is newly added, depends from claim 1 and adds that the proteinaceous substance are antibody fragments capable of specific binding with antigen, wherein the antibody fragments are F(ab) or F(ab')2 fragments. Support for the amendments may be found on page 6, lines 5-19, which state,

> "The term "biologically active" as used in the present description means that the target substances provided with this attribute have the functional properties desired or required for the respective purpose. If, for example, it is desired to produce antibodies, the protein produced, or a functional fragment thereof, is biologically active when it is capable of establishing the expected specific binding with the antigen."

Claim 24 is newly added, depends from claim 1 and adds that the proteinaceous substances are enzymes capable of converting a target substrate to a product. Support may be found at page 6, lines 5-19, which recite,

> "The term "biologically active" as used in the present description means that the target substances provided with this attribute have the functional properties desired or required for the respective purpose...For example, an enzyme is biologically active when it is capable of converting its target substrate."

Response to Rejection Under 35 USC § 103(a) (Obviousness)

I.

Claims 1-3 and 17 Are Not Obvious over Reutter et al In View of Lee et al.

Claims 1-3 and 17 stand rejected under 35 USC § 103(a) as being obvious over Reutter et al. (1996, Plant Tiss. Cult. BIotechnol. 1:142-147) in view of Lee et al. (US 6,020,169).

The examiner argues Reutter et al. teach growth of *P. patens* protonema transformed with a nucleic acid encoding a heterologous protein in a bioreactor culture and that the protonema produced large amounts of heterologous protein. The examiner acknowledges Reutter et al. do not disclose isolation of the protein from culture medium.

The examiner then cites Lee et al. as teaching isolation of biologically active heterologous protein from tobacco cells grown in suspension culture. The cells were transformed with a nucleic acid encoding Mab HC operably linked to a mammalian secretion signal peptide. The Mab HC was selectively secreted into the medium.

The examiner concludes the present invention is obvious because at the time of the invention it would have been obvious to modify the method of producing heterologus protein in *P. patens* protonema as taught by Reutter et al., to use a signal peptide in the transformation construct and isolate the protein from media as described in Lee et al.

A. Standard for Obviousness

A proper obviousness rejection requires consideration of the factual inquiries provided in Graham v. John Deere Co., 38 U.S. 1, 148 USPQ 459 (1966), including: 1) determining the scope and contents of the prior art; 2) ascertaining the differences between the prior art and the claims at issue; 3) resolving the level of ordinary skill in the pertinent art; and 4) considering the objective evidence of nonobviousness. Although Graham v. John Deere requires that certain factual inquiries be conducted to support a determination of the issue of obviousness, the actual determination of the issue requires an elevation in light of the findings in those inquiries as to the

obviousness *of the claimed invention as a whole*, not merely the differences between the claimed invention and the prior art. Lear Siegelr, Inc. v. Aeroquip Corp., 221 USPQ 1025, 1033 (Fed. Cir. 1984). Further, the teachings of a prior art reference are underlying factual questions in the obviousness inquiry. Para-Ordnance Mfg., Inc. v. SGS Imp. Int'l, Inc. 73 F.3d 1085, 1088 (Fed. Cir. 1995). Still further, a reference may be said to teach away when a person of ordinary skill, upon reading the reference, would be discouraged from following the path set out in the reference, or would be led in a direction divergent from the path that was taken by the applicant. In re Gurley, 27 F.3d 551, 553 (Fed. Cir. 1994). Accordingly, one can not use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention. In re Fritch, 23 USPQ2d 1780, 1784 (Fed. Cir 1992).

B. With respect to all claims, Lee et al. abandons the technical approach of using intact plants for obtaining heterologous polypeptides in favor of a divergent approach, namely using a cell-based system, and thus would discourage the skilled artisan from applying Lee et al. to an intact plant system

The examiner argues it would have been obvious to modify the method as taught by Reutter et al to use a signal peptide and isolate protein from media as described in Lee et al; however, this combination directly conflicts with the teachings of Lee et al., which expressly abandon the use of intact transgenic plants. Instead, when considering Lee et al. as a whole, it is apparent that the skilled artisan is discouraged from pursuing systems including intact transgenic plants but is encouraged to use cell-based systems, which include tobacco cells in suspension.

The use of intact transgenic plants for the production of foreign proteins is not new to Lee et al. In fact, Lee et al. acknowledge such systems can produce high levels of protein. However, while Lee et al. recognize the ability to produce high levels of protein in transgenic plants, Lee et al. also recognize that there exits significant challenges in obtaining purified protein from such systems. Specifically, purification of foreign protein from endogenous protein remains complex and expensive. This is demonstrated, for example, at col. 17, l. 67 through col. 18, l. 9,

"High-level production of functional foreign proteins has already been achieved in

> intact transgenic plants...However, the major drawback in using transgenic plants for production of large quantities of pure protein has been the cost of purifying the recombinant foreign protein away from endogenous plant proteins[.]"

The above concept was thoroughly considered in Lee et al. For instance, Lee et al. also noted antibody production was possible in plants. However, Lee et al. also noted that antibodies accumulate within the plant. Thus, while methods have been developed to efficiently produce proteins there remains significant challenges in efficiently purifying them. This is summarized at col. 1, ll. 56-59, which states,

> "Intact functional antibodies can be produced intracellularly in transgenic plants but intracellular accumulation requires expensive purification of antibodies from other cellular proteins."

Lee et al. also considered studies performed to produce functional IgM. Although use of a leader sequence in plants was used, again accumulation of the antibody remained in the plant. Referring to col. 1, ll. 66-67, "Again, accumulation of the multimeric antibody occurred only intracelluarly within the leaf tissue." Thus, while Lee et al. considered the use of intact transgenic plants, Lee et al. concluded purification from these systems remained complex and expensive.

Instead of developing a system for intact plants as previously proposed, Lee et al. abandoned the technical approach taken by others in favor of an entirely new and divergent approach. Specifically, Lee et al. abandoned the use of intact transgenic plants in favor of a cell-based system, which relied on cultured tobacco cells in suspension. The shift in technical approach is summarized, in part, at col. 4, ll. 34-40, which provides,

> "Plant suspension culture systems provide significant advantages over protein production in intact transgenic plants, which requires cultivation, harvesting and expensive extraction procedures to obtain non-secreted foreign proteins. Plant cell culture provides distinct advantages over other expression systems in terms of the purification of foreign polypeptides. Plant cells can be grown on an inexpensive defined medium lacking any polypeptide or other material that would complicate the purification of the secreted polypeptide or interfere with its biological activity. Moreover, the cultured plant cells appear to secrete few proteins into the culture medium. Also, high levels of contamination by proteins and other substances (e.g., endotoxin in the case of expression in E. coli) encountered in other expression systems, e.g., in the purification of polypeptides produced

intracellularly after disrupting cells to release the polypeptide, are avoided."

Thus, while the examiner argues the approach of Lee et al. should be combined with Reutter et al., it should be apparent that Lee et al. specifically abandoned an approach using intact transgenic plants. Thus, the combination itself is contrary to the teachings of Lee et al. Further, in view of the difficulties enumerated throughout Lee et al. with respect to systems using intact transgenic plants, one skilled in the art would be discouraged from following such a path.

While Lee et al. discourages the artisan to pursue a path including intact transgenic plants, Lee et al. purports to describe the successes achieved once adopting the new cell-based approach. Specifically, once adopting the new cell-based approach, Lee et al. reports the ability to secrete mouse heavy chain (Mab HC) from a tobacco cell suspension. This is demonstrated in example 1 and is referenced by the examiner. Thus, while Lee et al. first considered technical problems with isolation of protein from intact transgenic plants, once the technical approach was abandoned and the new approach adopted, Lee et al. reported secretion of Mab HC was successful. As such, success was only reported after adopting a cell-based system. Thus, in addition to discouraging the skilled artisan from pursuing a technical approach using an intact transgenic plant, Lee et al. clearly encourages or leads the skilled artisan to pursue the tobacco cell suspension approach, which purports to show positive results.

In summary, central to the teaching of Lee et al. is the abandonment of the traditional use of intact transgenic plants for heterologous protein production. Instead, Lee et al. provide a new technical approach, which includes the use a cell-based system. Once adopting this new approach, Lee et al. reported the ability to secrete Mab HC into the medium. Accordingly, when read as a whole, it should be apparent that Lee et al. would discourage the skilled artisan from pursuing an approach for production of heterologous protein using intact transgenic plants while concurrently leading or encouraging the skilled artisan to pursue an approach using a cell-based system, namely a tobacco cell suspension.

Thus, while Reutter et al. provide a method of producing large amounts of heterologous protein, Reutter et al. do not teach obtaining protein from the medium, without disrupting tissues

or cells. Further, while Lee et al. also considered the ability for high yielding protein production in intact plants, Lee et al. abandoned the technical approach to pursue a divergent path. Accordingly, given the teachings of Reutter et al. in view of Lee et al. the skilled artisan would be led away from an intact transgenic plant approach to pursue a cell-based system. Thus, a combination of an intact transgenic plant system or intact protonema with Lee et al. would be contrary to the explicit teachings of Lee et al. Accordingly, applicants respectfully request the rejections be withdrawn and the claims allowed.

C. With respect to claim 2, Lee et al. do not provide a biologically active protein but instead report the secretion of Mab HC, which is not associated with light chain to form a functional antibody

Claim 2 includes the limitation that the proteinaceous substances released into the culture medium are biologically active. Thus, one skilled in the art would understand that the limitation set forth in claim 2 would provide a functional proteinous substance; or a protein that performs its biological function. While the examiner cites Lee et al. as producing Mab HC, Lee et al. was not able to secrete an antibody that can target antigen and thus did not produce a proteinaceous substance, which is able to perform its biological function. Thus, in Lee et al. the Mab HC is not biologically active as understood by those skilled in the art.

Antibodies are part of the humoral immune system. They prevent pathogens from entering or damaging cells by binding to them; they stimulate the removal of pathogens by macrophages and other cells by coating the pathogen; and they trigger destruction of the pathogen. Thus, central to biological function of an antibody is the ability to recognize the pathogen or antigen. This is performed collectively through the use of light chain and heavy chain. Thus, to be biologically active one skilled in the art would understand that the antibody includes light chain and heavy chain.

Lee et al. recognized the importance of an antibody having both light chain and heavy chain for antigen binding. Although secretion of an intact antibody was attempted it was not successful. Instead, Lee et al. first describes the intact antibody was found in the apoplastic

space. Additional attempts were performed to secrete the intact antibody to the medium; however, in these studies the antibody was delivered to the choloroplast, which also remains within the cell. Thus, while Lee et al. recognized the requirement of both light chain and heavy chain for proper function, Lee et al.'s attempt at producing a functional antibody was unsuccessful and demonstrates significant difficulties in the secretion of proteinaceous substances having biological activity. This is summarized in col. 18, ll. 30-41,

> "Proper assembly of intact recombinant nascent antibody heavy and light chains in transformed tobacco plant cells requires the native or heterologous signal peptides[.] The signal peptide is required to target the antibody for secretion into the endoplasmic reticulum and through the plasma membrane into the apoplastic space. However, even though assembled antibody molecules appear to be secreted through the plasma membrane, they apparently cannot be efficiently secreted through the cell wall into the extracellular medium. As a result, the assembled antibody accumulates in the apoplastic space[.] An attempt to replace the native signal peptide of the murine antibody with a barley α-amylase signal peptide led to an unexpected mistargeting of the immunoglobulin chains to the chloroplast."

Although Lee et al. could not secrete functional antibody, Lee et al. did screen for the presence of Mab HC using a goat anti-mouse IgG and purified the Mab HC using a protein G column. Although these studies indicate the presence of Mab HC in the media, they do not demonstrate biological relevance or biological activity. In other words, while the ability to capture Mab HC was demonstrated, the biological activity of Mab HC was not demonstrated. This is consistent with the lack of light chain in a proper antibody complex.

Since Lee et al. do not teach a proteinaceous substance released into the culture medium that is biologically active, Lee et al. in view of Reutter et al. do not render the present invention obvious. Accordingly, applicant respectfully requests the rejection be withdrawn.

II.

Claims 1-3 and 17 Are Not Obvious over Reutter et al In View of Lee et al. and Further in View of Nasu et al.

Claims 1-3 and 17 stand rejected under 35 USC § 103(a) as being obvious over Reutter et al. (1996, Plant Tiss. Cult. Biotechnol. 1:142-147) in view of Lee et al. (US 6,020,169) further in view of Nasu et al (1997) J. Ferm. Bioengin. 58:519-523).

The examiner incorporates the discussion of Reutter et al in view of Lee et al as provided above and adds that Nasu et al. teach the transformation of *Marchantia polymorpha*, which is an auxotroph and thus its growth does not require sugars, vitamins or phytohormones.

The deficiencies of the rejection of Reutter et al in view of Lee et al. are discussed above. Nasu does not correct the deficiencies in the rejection. Broadly, Nasu involves the fixation of transformed cells in formaldehyde to assess intracellular expression of heterologous proteins and thus kills the cells.

Accordingly, claims 1-3 and 17 are not obvious over Reutter et al. in view of Lee et al. and further in view of Nasu et al. and applicant respectfully requests the rejection be withdrawn.

Respectfully submitted,

6/06/2009
Date

Raymond Wagenknecht
Reg. No. 50,948

Biotech Beach Law Group, PC
625 Broadway, Ste. 1210
San Diego, CA 92101
(619) 238-1179